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Comparative effectiveness and field persistence of insect growth regulators on a field strain of the cotton leafworm, *Spodoptera littoralis*, Boisd (Lepidoptera: Noctuidae)

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ABSTRACT

Toxicity effects and field persistence of the insect growth regulators lufenuron, flufenoxuron and triflumuron were assessed in the laboratory using second and fourth larval instars of *Spodoptera littoralis*. Laboratory bioassays indicated that lufenuron was more effective on both 2nd and 4th larval instars, as well as killing both larval instars faster than flufenoxuron or triflumuron. Field-laboratory experiments were conducted to show direct and residual effects of the tested IGRs in terms of toxicity and stability. They indicated that all the tested insecticides were stable under field conditions and give high percentages of mortality. Overall, lufenuron was more efficient than the other tested insecticides. In addition, it gave a faster kill in some testing periods. Data presented in this work show greater efficiency of lufenuron in controlling *S. littoralis* than flufenoxuron or triflumuron. Using this insecticide for cotton leafworm control in cotton fields may give better results under field condition.

1. Introduction

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is an important and widespread pest of cultivated crops primarily in tropical and subtropical regions (Brown and Dewhurst, 1975). Because of its polyphagy, this species causes economical yield losses on several crops (Carter, 1984). Considerable damage is recorded regularly on cotton, spinach, alfalfa, pepper, eggplant, tomato, lettuce, bean, strawberry and some ornamental crops. *S. littoralis* has been recorded throughout Africa, the Middle East, in the Mediterranean basin, Asia, and Europe (Bayoumi et al., 1998; El-Aswad et al., 2003; Pineda et al., 2007). In addition to the direct damage caused by reducing photosynthetic area, the occurrence of larvae, feeding damage and excrement reduce marketability of vegetables and ornamentals (Pluschkell et al., 1998).

The intensive use of broad-spectrum insecticides against *S. littoralis* has led to the development of resistance to many registered pesticides use for its control (Aydin and Gürkan, 2006; Smagghe et al., 1999). Recently in Egypt, the use of conventional insecticide applications during the period when egg-masses predominate is not recommended to conserve natural enemy populations. Meanwhile, the use of the insect growth regulators (IGR_S) is considered as a possible alternative for controlling the newly hatched larvae (Raslan, 2002).

Insect growth regulators (IGRs) are compounds that can regulate the growth of insect pests and play an important role in integrated pest management systems (Kai et al., 2009). They are also called third-generation insecticides, as they disrupt the normal activity of the endocrine system of insects, affecting development, reproduction or metamorphosis of the target insects. IGRs include juvenile hormone (JH) mimics and chitin synthesis inhibitors (CSIs) (Hoffman and Lorenz, 1998; Tunaz and Uygun, 2004). They have a slower action than neurotoxic synthetic chemical insecticides. CSIs, such as hexaflumuron, lufenuron and diflubenzuron, inhibit the production of chitin, a major component of the insect exoskeleton. Insects treated with CSIs become unable to synthesize new cuticle, and therefore are unable to successfully molt into the next stage. It was originally thought that insects would be unable to develop resistance to molecules that mimic their own hormones, but there is already evidence of resistance developing to several IGRs, including kinoprene, pyriproxifen and diflubenzuron. Resistance seems to result from decreased penetration and increased metabolism of the compound (Hoffman and Lorenz, 1998).

The aim of this work was to evaluate the susceptibility of second (2nd) and fourth (4th) instar larvae of a field strain of the cotton leafworm, *S. littoralis* (Boisd.) to lufenuron, flufenoxuron and triflumuron as well as their persistence under field condition.

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Table 1
Characteristics of insecticides used in the present study.

Names		Formulations[a]	Mode of action	Chemical group	Recommended rate (ml/ha)	Parent company
Technical	Commercial					
Lufenuron	Luferon	EC, 5%	CSI[b]	IGR[c]	380	Agrochem
Flufenoxuron	Ageron	DC, 10%	CSI	IGR	476	Agrochem
Triflumuron	Alsystin	SC, 48%	CSI	IGR	238	Bayer cropScience

[a] EC: Emulsifiable concentrate, DC: Dispersible Concentrate and SC: Suspension concentrate.
[b] CSI: chitin synthesis inhibitor and also names as insect development inhibitors.
[c] IGR: insect growth regulator.

2. Materials and methods

2.1. Insect colony and rearing

Egg-masses of a field strain of the cotton leafworm *S. littoralis* (Boisd.) were collected from a cotton field near El-Kinayate City, Sharkia Governorate, Egypt. The collected egg-masses of the cotton leafworm were reared in the laboratory at 27 ± 2 °C and 65 ± 5% R.H. for one generation on clean untreated cotton leaves to get the number needed in the study, as described by El-Defrawi et al. (1964). Briefly, 50-60 pupae that resulted from egg-masses collected from field were placed in wide glass gars until adult emergence. The emerged adults were provided with blotting paper or branches of Tafla (*Nerium oleander*) for adult oviposition, and were supplied with 10% honey-water on a cotton wick as a food source for the moths. Once adults emerged (5–6 days after pupation) and oviposition had begun, the blotting paper or Tafla leaves where eggs had been laid were removed every 2 days, soaked for 5 min in 5% formaldehyde, and rinsed for 5 min in running distilled water. The blotting paper or Tafla leaves were then placed on paper toweling and dried overnight. The following day, egg-masses were placed in a container provided with cotton leaves, and the rearing procedure was repeated as described above until they reached the required instars.

2.2. Tested insect growth regulators

Commercial formulations of the insecticides lufenuron 5% EC (Luferon), flufenoxuron 10% DC (Ageron) and triflumuron 48% SC (Alsystin) were used in this study (Table 1).

2.3. Laboratory bioassay

All of the insecticide formulations were prepared to obtain the proper concentrations using tap water. Series of five to six concentrations ranging from 0.01 to 10 ppm of each insecticide were used so that LC_{50} and LC_{90} values could be calculated. The concentrations studied were prepared originally from a stock solution of every insecticide which was further diluted with tap water to get the desired concentrations. Second and 4th larval instars of the field colony were used for laboratory bioassays. Cotton leaves were cut by pressing with a cutter ring which had a sharp-cut edge to provide disks of 65 mm diameter and then dipped for 30 s in each concentration. After dipping, disks were dried for ~30 min in the air under room temperature. Treated disks were transferred to 16 OZ clear plastic cups with perforated snap-fit caps (Bio-Serv, Frenchtown, NJ) and 10 larvae of 2nd or 4th instars were used/concentration. Every concentration was replicated three times. All replicates were reserved at 27 ± 2 °C and 65 ± 5% R.H. The larvae were allowed to feed on treated leaves for 24 h, then for 5 days on fresh untreated leaves. Ten larvae of the same instar were kept on untreated cotton leaves for the control and replicated three times. Thereafter, mortality counts were started 24 h after treatment and observed at 24 h intervals. Mortalities were corrected using the formula of Abbott (1925) at the end of 5 days after treatment. Concentration mortality regression lines were fitted using the method adopted by Finney (1971).

2.4. Time-mortality relationship

High concentrations of the tested IGR insecticides were used to study the time-mortality relationship. Insecticide concentrations of 10, 100 and 1000 ppm were prepared with tap water as previously described and the same procedure previously mentioned was used for preparing cotton leaf disks treated at the tested concentrations. Ten larvae of 2nd or 4th instars were introduced to one disk/concentration in 16 OZ clear plastic cups with perforated snap-fit caps. Every concentration was replicated three times. Mortality was recorded after 24 h of treatment and at 12 h intervals for up to 5 days.

2.5. Field efficiency and persistence of the tested IGRs

For the field persistence study, field cotton (0.5 ha) located in El-Kinayate, Sharkia Governorate, Egypt was divided into plots (4 m^2 each) and labeled. Insecticides were sprayed using a Motorized Knapsack Sprayer (with single cylinder 2-cycle engine, 2.6 KW/3.5 HP power, 108 dB pressure and 20 L tank capacity). The tested insecticides applications were done on three successive plots according to the field rate of application/insecticide recommended by Egyptian Ministry of Agriculture (Table 1) separated by untreated plots to prevent cross contamination. Three untreated plots were reserved as controls. About 2 h after application, leaves of every plot were collected randomly and put in paper bags then transferred to the laboratory for bioassay to determine the direct effects of the tested insecticides. Ten larvae of 2nd or 4th larval

Table 2
LC_{50} and LC_{90} values of toxicity effects of lufenuron, flufenexuron, and triflumuron tested on 2nd larval instar of *S. littoralis*.

Insecticides	LC_{50} (95% CL)[a]	LC_{90} (95% CL)	Slope		Heterogeneity (X^2/df)	Relative potency[b]	
			Mean	SE		LC_{50}	LC_{90}
Lufenuron	0.02 (0.01–0.03)	1.22 (0.65–2.84)	0.7	0.06	3.2	1	1
Flufenexuron	0.05 (0.03–0.08)	3.66 (1.86–10.09)	0.6	0.06	4.9	2.5	3.0
Triflumuron	0.19 (0.14–0.26)	7.04 (3.95–15.40)	0.9	0.09	4.7	9.5	5.8

[a] LC_{50} or LC_{90} and 95% fiducial limits (CLs) are given in ppm of a.i.
[b] Relative potency is calculated as LC_{50} or LC_{90} of the tested insecticides/LC_{50} or LC_{90} of the most effective insecticide.